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Morphometric variation of the oriental river prawn (*Macrobrachium nipponense*) in Taiwan

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ABSTRACT

Morphometric differences were used to elucidate the stock geographic variations and phylogeography of *Macrobrachium nipponense* in Taiwan. Eight samples were collected from three estuaries (Tamsui River Estuary [TSE], Kaoping River Estuary [KPE], and Houlung River Estuary [HLE]) and five reservoirs (Shimen Reservoir [SMR], Mingde Reservoir [MDR], Deji Reservoir [DJR], Tsengwen Reservoir [TWR], and Chengqing Lake Reservoir [CLR]). Twelve morphometric measurements were size-standardized by the allometric method and via Cluster Analysis and Canonical Variate Analysis (CVA). Randomisation tests were used to verify the morphometric variation between groups. The results clustered the eight samples into a minimum of three groups. The first group included four reservoir samples (i.e. DJR, MDR, CLR, and TWR); the second included the SMR sample, and the third comprised the remaining estuarine samples (i.e. TSE, HLE, and KPE). Morphometric variation among the three groups was significant for each sex. Significant differences between these three groups may be derived from evolutionary origins, geographic events or environmental adaption which was discussed in the paper. The difference between multivariate allometric coefficients in both sexes and sites were also tested based on the eight group data sets, and the result showed that the difference between sexes was significant.

Introduction

In order to effectively manage fishery resources, identification of the population structure of an explored species is necessary (Grimes et al., 1987). Morphometric variability can be used to explore differences among geographically distinct populations; it was also used to attribute distinct genetic structures or environmental conditions to each geographic location (Kinsey et al., 1994). A previous study showed that environmental variation leads to differences in population structure, including morphological heterogeneity (Tzeng et al., 1998; Begg et al., 1999; Tzeng and Yeh, 1999; Giri and Collins, 2004; Collins et al., 2007; Giri and Loy, 2008; Torres et al., 2014). Therefore, morphometric variation between stock can provide a basis for stock structure, and might be more applicable for studying a short-term, environmentally induced variation (e.g. in fisheries management) (Begg et al., 1999).

The oriental river prawn (*Macrobrachium nipponense*) is native to and broadly distributed throughout East Asia (i.e. China, Japan, Korea, Vietnam, Myanmar, and Taiwan) (Yu and Miyake, 1972; Cai and Ng, 2002); it has been introduced to Singapore, the Philippines (Cai and Shokita, 2006), Uzbekistan (Mirabdullaev and Niyazov, 2005), southern Iraq (Salman et al., 2006), and Iran (De Grave and Ghane, 2006). In Taiwan, it has a wide distribution covering almost the entire island. This species was assumed to have originated in mainland China, and subsequently, dispersed to Taiwan through the land bridges between Taiwan and China during the Pleistocene period (Chen et al., 2009). The oriental river prawn is a non-obligatory amphidromous prawn (Shy et al., 1987, 1996; Mashiko and Shy, 2008). A number of oriental river prawns remain in the estuaries to complete their life cycle, whereas some populations are found in inland freshwater lakes because of a shift in habitat from estuaries to inland freshwaters (Mashiko, 1990; Tzeng et al., 1998; Mashiko, 2000; Mashiko and Numachi, 2000).

Spatially disconnected river systems impose unique natural geographical constraints on freshwater fauna (Avise, 2000). The

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dispersal of freshwater species is usually limited and dependent on the continuity of drainage basins, including geographic barriers, changes in hydrology, drainage rearrangements, connections between drainages, and sea level changes (Bilton et al., 2001). In particular, several rivers that were separated by the sea later became connected to one another during the glacial period. These connected waterways can facilitate the migration of freshwater fauna between river systems. Therefore, geologic events have considerably influenced endemism and the population structure of freshwater fauna (Shy et al., 1996; Mashiko, 2000; Mashiko and Numachi, 2000; Mashiko and Shy, 2008; Chen et al., 2009).

Historically, geographic events have been shown to affect the demographic distributions of freshwater fauna more than of terrestrial species (Kotlik and Berrebi, 2001). Studies on the population genetic structure of marine biota have frequently indicated that organisms with high dispersal capacity have limited genetic distinction over a large geographic area (Hellberg, 1996; Tzeng, 2004). These studies suggest morphometric variability derived from gene flow between populations.

The morphological features of an organism are not autonomous and changes in various aspects of morphology are coordinated (Zelditch et al., 1992). Consequently, unless a specific morphometric measurement is known to have a genetic foundation, morphology is best described by using multivariate techniques (Thorpe and Leamy, 1983). Most of the variability in a set of multivariate measurements is derived from size (Junquera and Perez-Gandaras, 1993; Anastasiadou et al., 2009). Thus, shape analysis should be free from the effect of size to avoid misinterpretation of the results (Strauss, 1985). Several univariate and multivariate techniques can be used to remove the effect of size (e.g. regression analysis and multiple group principal component analysis [MGPCA]). These allometric techniques can be used to adequately achieve size and shape separation and reasonably meet statistical assumptions (Reist, 1985).

Population genetic and morphometric variation were compared among different populations of oriental river prawns in different habitats ranging from the estuarine to fresh-water ecosystems of Japan (Mashiko and Numachi, 2000). They indicate that populations from fresh-water are differentiated from estuarine populations in genetic content and morphometric traits. However, there is still no information on the stock structure and morphometric characteristics of oriental river prawn populations in Taiwan. Therefore, the objective of the present paper was to use multivariate statistical techniques to analyse and compare the size-free shape of different geographic oriental river prawn populations in Taiwan, to elucidate the stock structure of this species.

Materials and methods

Sample collection

Eight samples were collected from three estuaries (i.e. Tamsui River Estuary [TSE], Kaoping River Estuary [KPE], and Houlung River Estuary [HLE]) and five reservoirs (i.e. Shimen Reservoir [SMR], Mingde Reservoir [MDR], Deji Reservoir [DJR], Tsengwen Reservoir [TWR], and Chengqing Lake Reservoir [CLR]) between December 2013 and January 2014 (Fig. 1). The shrimp cage belongs to cage

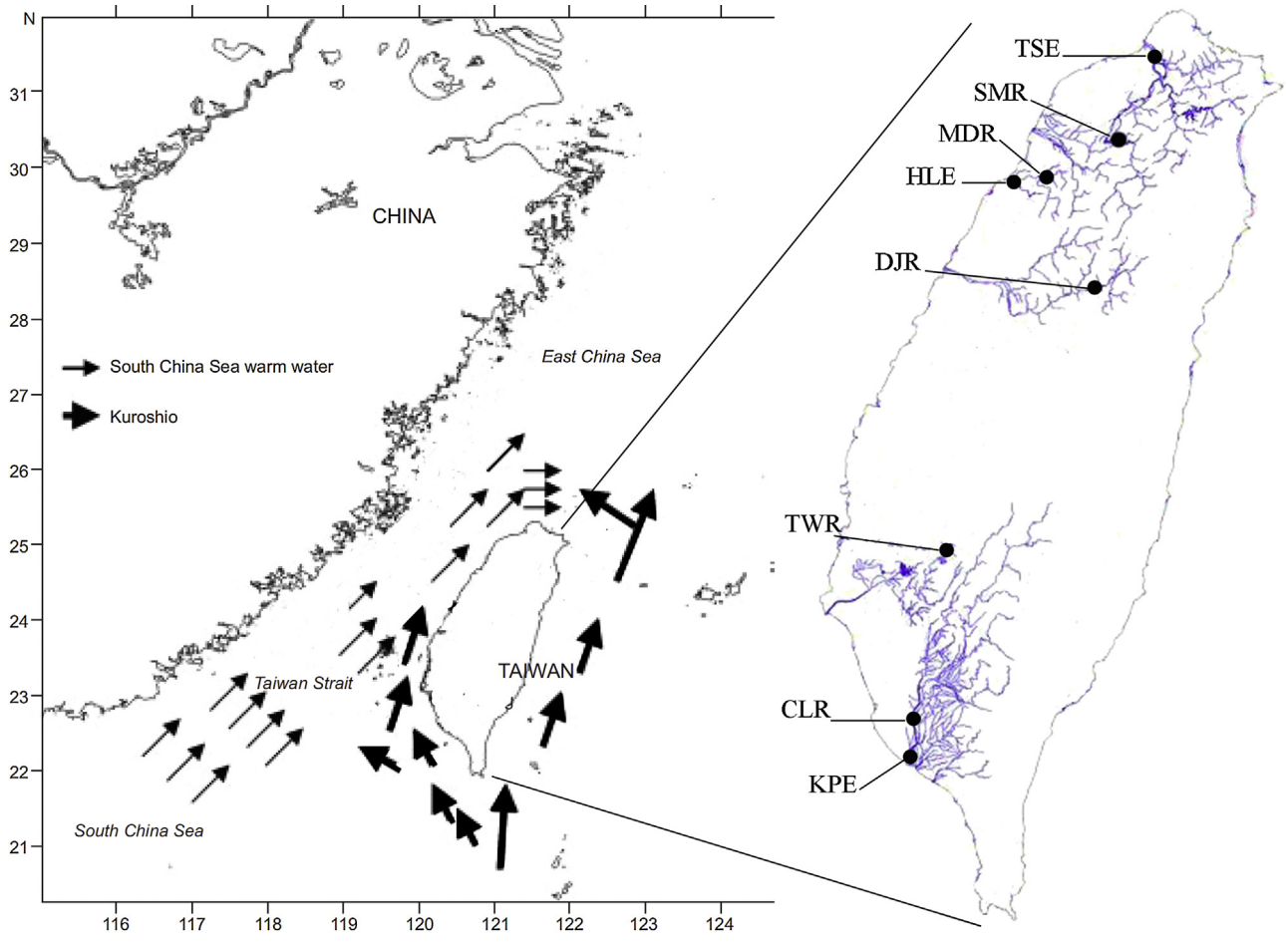

Fig. 1. Shadowed areas show the sampling areas from Taiwan.

Table 1
Code of sampling site, sample size, sampling date and mean, standard deviation (SD) and range of carapace length (CL) for male and female *Macrobrachium nipponense* in Taiwan.

Area code	Sampling site	*n*	Sex	Sampling date	CL (mm)	
					Mean (SD)	Range
TSE	Tamsui river estuary	50	f	January 2014	16.55 (2.74)	12.11–21.69
		51	m		19.89 (3.25)	14.84–26.50
HLE	Houlung river estuary	55	f	January 2014	17.13 (3.06)	12.54–22.80
		51	m		20.20 (3.62)	14.7–31.13
KPE	Kaoping river estuary	56	f	January 2014	17.11 (3.04)	12.54–22.80
		56	m		18.35 (3.41)	11.85–25.22
SMR	Shimen reservoir	56	f	January 2014	17.51 (2.63)	12.33–22.39
		55	m		19.60 (2.87)	14.02–25.69
MDR	Mingde reservoir	54	f	January 2014	17.50 (2.48)	12.50–22.39
		57	m		19.55 (3.64)	13.59–26.42
DJR	Deji reservoir	50	f	December 2013	16.29 (2.61)	12.37–21.16
		55	m		19.76 (3.68)	14.35–27.24
TWR	Tseng Wen reservoi	55	f	December 2013	18.59 (2.18)	13.10–22.61
		65	m		22.94 (3.99)	14.2–29.83
CLR	Chengqing Lake reservoir	50	f	January 2014	16.29 (2.21)	12.46–21.85
		57	m		18.54 (3.05)	14.12–28.65

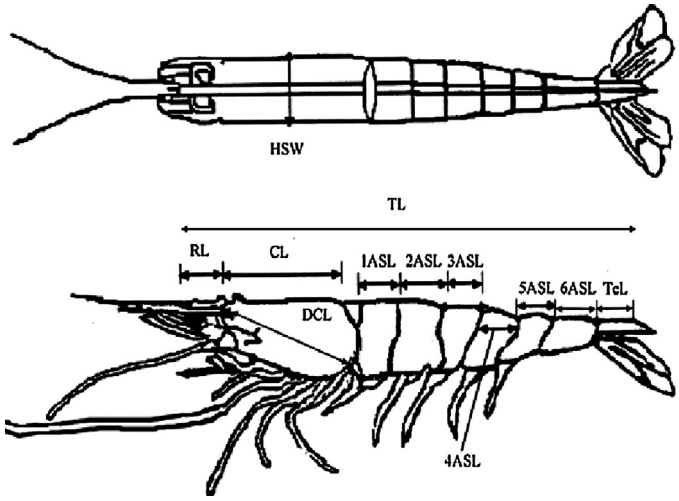

Fig. 2. Diagram of *Macrobrachium nipponense* showing the body parts measured.

traps. Collecting was performed with 5.0 mm-mesh trap nets and 5.5 mm-mesh dip nets (Mashiko, 2000). The sex was identified and individuals were separated accordingly. All samples were measured in fresh conditions. Twelve morphometric measurements were assessed for each specimen (Fig. 2), including hepatic spine width, Rostrum length, carapace length, diagonal carapace length, first abdominal segment length, second abdominal segment length, third abdominal segment length, fourth abdominal segment length, fifth abdominal segment length, sixth abdominal segment length, telson length, and total length. The ranges of carapace lengths of the samples were restricted to specific length classes in the present study. The sample size, sampling area code, and the mean and range of the carapace length are summarised in Table 1. Then, we performed a two-way analysis of variance (ANOVA) to test if they were significant differences between sexes, sites and interaction in carapace length. A total of 873 individuals were measured in this study. All measurements were rounded to the nearest 0.01 mm.

Morphometric variation analyses

The allometric equation $Y = aX^b$ was used to remove the effect of carapace length (X) variation on characteristic length (Y) in each sample (Reist, 1985; Tzeng et al., 1998; Tudela, 1999; Tzeng, 2004; Paramo and Saint-Paul, 2010). It standardizes all characteristics according to the following equation:

$$Y_i^* = Y_i \left[\frac{X_i}{\bar{X}} \right]^b \quad (1)$$

where Y_i^* is the standardised measured length of the ith specimen; Y_i is the measured length of the ith specimen; X_i is the measured carapace length of the ith specimen; and $\bar{X}$ is the mean value of the carapace lengths of the specimens examined. Correlation coefficients between each pair of characters before and after size effect removal were checked. In such a case it is expected that the absolute value of correlation coefficients would decrease after size effect removal (Murta, 2000). The resulting measurements were subjected to canonical variate analysis (CVA) and unweighted pair-group method with arithmetic means (UPGMA).

A dendrogram of the eight samples was constructed by UPGMA by using Mahalanobis distances between population centroids to assess the degree of similarity between the samples (Sneath and Sokal, 1973; Tzeng, 2004; Rohlf, 2007; Torres et al., 2014). The Mahalanobis distance was chosen because it is invariant to differences in scale among variables (Dryden and Mardia, 1998).

Canonical variate analysis, a linear ordination technique, was also performed to discriminate among samples. The coefficients of the linear combination obtained through maximising the ratio of the inter- to intra-group variance defined the canonical vectors (Owen and Chmielewski, 1985; Tzeng, 2004; Torres et al., 2014). After the CVA was conducted, the first two canonical scores were plotted, and the confidence levels for the observed samples were also shown via 95% confidence ellipse around the mean of each sample (Owen and Chmielewski, 1985; Tzeng and Yeh, 2002).

To test the significance level of the morphometric differences between different groups derived from cluster analysis and CVA, a randomisation test was performed (Solow, 1990; Tzeng and Yeh, 1999, 2002). All specimens were randomly assigned to one of two groups. The new dataset was then analysed by multivariate discriminant analysis, and the cross-validation estimator (Pc) was estimated (Solow, 1990). This estimator measures the proportion of individuals that are misclassified. Resampling was performed 1000 times, with a different random permutation for each analysis. This randomisation test assesses the significance of the misclassification rate by comparing the proportion of individuals (Po) that have been misclassified in the original dataset to the proportion misclassified (Pc) in each randomised dataset. The proportion (P) of the observed Pc that was $\leq$Po was calculated. This P-value can be interpreted in the same way as that of conventional tests of

Table 2
Correlation coefficients between characters, before and after the removal of the size effect, are respectively shown below and above the diagonal.

(A)Female

Variable	HW	RL	DCL	1PL	2PL	3PL	4PL	5PL	6PL	TeL	TL
HW		0.10	**0.39**	−0.08	−0.16	0.11	−0.01	−0.11	0.06	0.30	0.07
RL	0.67		**0.17**	−0.21	−0.12	−0.20	−0.17	−0.29	−0.09	0.06	−0.12
DCL	**0.79**	**0.78**		0.02	0.05	0.02	−0.08	−0.08	0.11	**0.31**	0.12
1PL	0.36	0.36	0.45		**0.60**	0.47	0.41	0.39	0.18	0.19	0.21
2PL	0.32	0.43	0.48	**0.71**		0.49	0.40	0.48	0.31	0.23	0.24
3PL	0.44	0.33	0.44	0.61	0.61		0.40	0.32	0.31	0.19	0.12
4PL	0.30	0.26	0.30	0.53	0.52	0.51		0.39	0.18	0.07	0.16
5PL	0.29	0.28	0.36	0.54	0.61	0.47	0.51		0.27	0.09	0.23
6PL	0.52	0.51	0.60	0.45	0.55	0.52	0.38	0.47		0.19	−0.03
Tel	0.70	0.63	**0.73**	0.49	0.52	0.48	0.33	0.39	0.57		0.23
TL	0.53	0.58	0.62	0.49	0.53	0.39	0.39	0.48	0.43	0.60	

(B)Male

Variable	HW	RL	DCL	1PL	2PL	3PL	4PL	5PL	6PL	TeL	TL
HW		0.17	**0.30**	−0.16	−0.12	0.00	−0.05	−0.09	0.00	0.12	0.03
RL	0.69		**0.23**	−0.24	−0.26	−0.08	−0.12	−0.20	0.06	**0.10**	−0.30
DCL	**0.76**	**0.81**		−0.09	−0.07	0.04	−0.01	0.02	0.04	**0.13**	−0.02
1PL	0.29	0.36	0.45		**0.73**	0.56	0.55	0.43	0.23	0.28	0.28
2PL	0.34	0.36	0.47	**0.81**		0.57	0.48	0.45	0.29	0.25	0.30
3PL	0.43	0.43	0.53	0.68	0.68		0.59	0.34	0.40	0.32	0.22
4PL	0.34	0.42	0.47	0.68	0.63	0.69		0.34	0.27	0.26	0.31
5PL	0.38	0.42	0.51	0.59	0.62	0.55	0.54		0.31	0.09	0.35
6PL	0.51	0.57	0.61	0.50	0.53	0.64	0.52	0.55		0.26	0.16
Tel	0.67	**0.76**	**0.78**	0.56	0.55	0.59	0.54	0.50	0.65		0.16
TL	0.56	0.59	0.66	0.55	0.58	0.51	0.57	0.60	0.56	0.68	

significance: if it is <5%, then this provides some evidence that the morphometric difference between the two groups is significant; if it is <1%, then it provides strong evidence that the morphometric difference between the two groups is significant (Solow, 1990).

All analyses in the present study were performed using the Statistical Analysis System software (SAS, 2014).

Results

Correlation coefficients between characters before and after size effect removal are shown in Table 2. Most coefficients were highly significant before size effect removal and were considerably reduced after. Therefore, all data used in following analyses is almost free from size effect.

Dendrograms of the eight samples for females and males are shown in Figs. 3 and 4. The results for females and males were very similar. The eight samples were clustered into two groups. The first group included all samples from the reservoirs (i.e. DJR, MDR, CLR, and TWR), except SMR; the second group included the SMR, TSE, HLE, and KPE samples. The second group might be further divided into two subgroups. The first subgroup included SMR; the second subgroup included all three estuarine samples. Therefore, at least three clusters were identified among the eight samples.

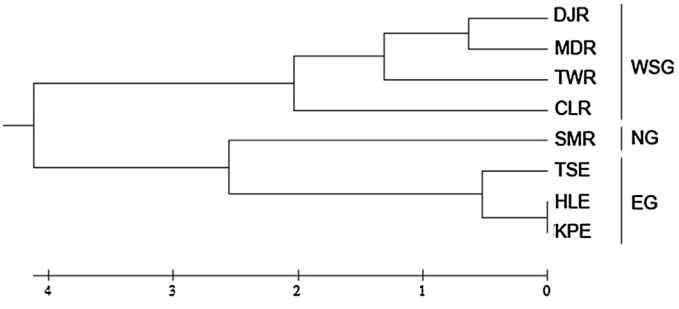

Fig. 3. Dendrogram for eight sampling areas for female data set, WS-reservoir group (WSG), N-reservoir group (NRG), and Estuarine group (EG).

The first two eigenvectors and the percentages of total variance explained by the first two eigenvalues obtained from CVA for each sex are shown in Table 3. The majority of total variance was explained by the first two canonical variables for each sex. The first eigenvalue accounted for 67 and 81% of the total variance for

Table 3
The first two eigenvectors and percentages of total variance explained by the first two eigenvalues obtained from canonical variate analysis.

(A)Female

Variable	First eigenvector	Second eigenvector
Hepatic spine width	−0.25	0.82
Rostrum length	−0.13	0.33
Diagonal carapace length	−0.31	−0.27
First abdominal segment length	−0.08	0.50
Second abdominal segment length	0.17	−0.03
Third abdominal segment length	0.25	0.04
Fourth abdominal segment length	0.37	0.19
Fifth abdominal segment length	0.20	−0.46
Sixth abdominal segment length	−0.34	−0.21
Telson length	−0.15	−0.09
Total length	0.89	0.16
Eigenvalue	1.79	0.42
Percentage variance	**67%**	**15%**

(B)Male

Variable	First eigenvector	Second eigenvector
Hepatic spine width	−0.23	0.21
Rostrum length	−0.17	−0.15
Diagonal carapace length	−0.50	0.39
First abdominal segment length	0.00	0.26
Second abdominal segment length	0.18	−0.16
Third abdominal segment length	−0.06	−0.39
Fourth abdominal segment length	0.19	−0.58
Fifth abdominal segment length	0.12	−0.02
Sixth abdominal segment length	0.05	0.77
Telson length	−0.13	−0.37
Total length	0.97	0.22
Eigenvalue	3.47	0.33
Percentage variance	**81%**	**8%**

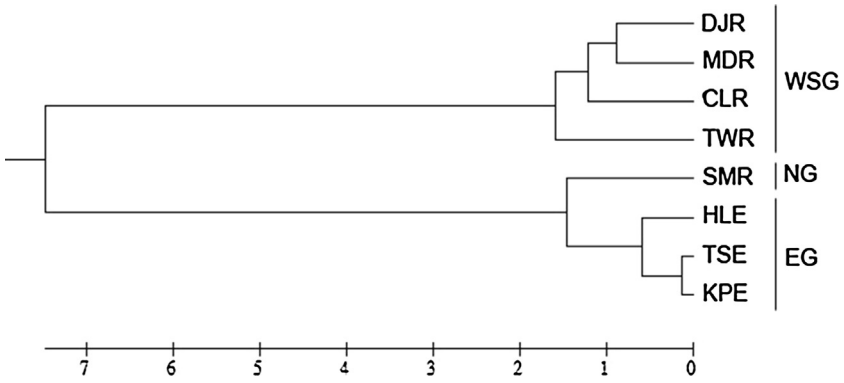

Fig. 4. Dendrogram for eight sampling areas for male data set, WS-reservoir group (WSG), N-reservoir group (NRG), and Estuarine group (EG).

females and males, respectively; the second eigenvalue accounted for 15 and 8% of the total variance for females and males, respectively. The measurements of primary importance in the first and second eigenvectors were the total length (0.89) and the Hepatic spine width (0.82) and the total length (0.97) and the sixth abdominal segment length (0.77) for females and males, respectively. The plotting of 95% confidence ellipses around the means of the first two canonical scores for each sex is shown in Fig. 5. The results for females and males were very similar. The eight samples were

Table 4
Two-way ANOVA (sex×site) of carapace size measured at eight study sites in *Macrobrachium nipponense*.

	df	*F* value	*p* (*F*)
Sex	1	167.78	<0.001
Site	7	12.47	<0.001
Sex × site	7	2.90	0.005

If the results of an ANOVA indicated significant treatment effects at the 0.05 probability level (*p*).

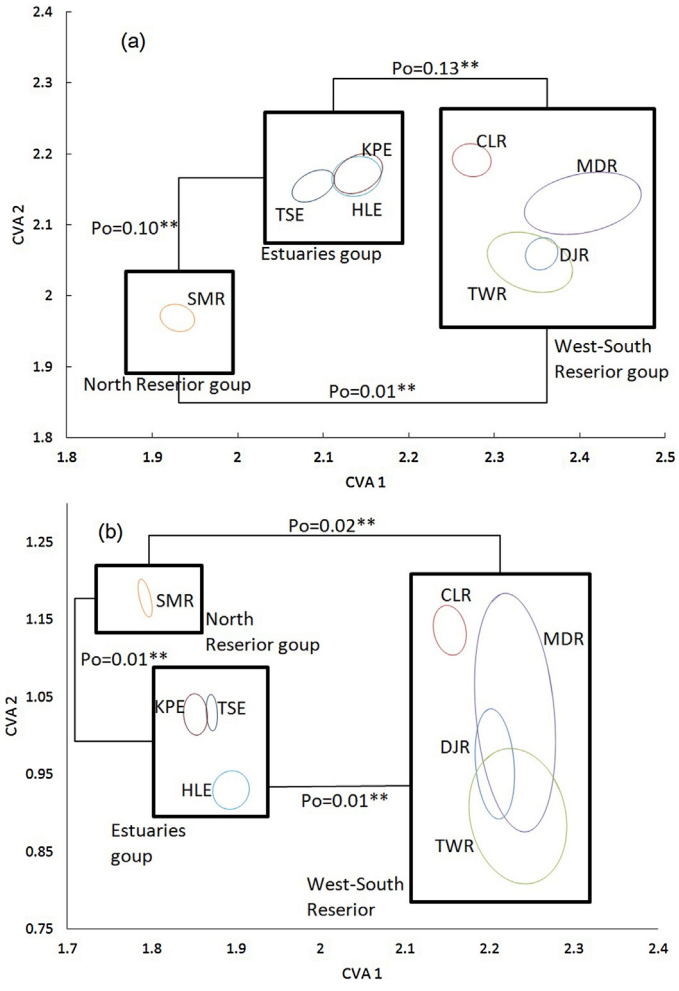

Fig. 5. Plot of 95% confidence ellipses around sample from (a) female and (b) male spreads and group means of first two canonical scores, the WS-reservoir group (WSG), the N-reservoir group (NRG), and the Estuarine group (EG).

clustered into at least three groups. The WS-reservoir group included four reservoir samples (i.e. DJR, MDR, CLR, and TWR); the N-reservoir group included the sample from SMR; and the estuarine group included all estuarine samples (i.e. TSE, HLE, and KPE).

Based on the results of the cluster analysis and CVA, the individuals from the TSE, HLE, and KPE samples (i.e. Estuarine group, EG) and specimens from the DJR, MDR, CLR, and TWR samples (i.e. West-South-reservoir group, WSG) were separately pooled as two new datasets for each sex. The individuals from SMR (i.e. North-reservoir group) were regarded as a single dataset, which was also analysed in a randomisation test for each sex. Besides, all populations exhibited allometric relationships between the carapace length and had a statistically significant relationship. The carapace length was significantly different between sexes and sites (Table 4).

The Po values between the West-South Reservoir groups, Estuarine groups, and between the Estuarine and North Reservoir groups for females were 0.13–0.01, respectively (Fig. 6a–c). The Po values between the WS-reservoir and Estuarine groups and between the Estuarine and N-reservoir groups for males were 0.14–0.02, respectively (Fig. 6d–f). All results from the randomisation tests were significant ($P = 0$), which indicates that it is unlikely that the extremely low misclassification rates were a result of chance alone and morphometric differences between the three groups were significant.

Discussion

The findings suggest that the eight samples into a minimum of three groups. The first group included the TSE, HLE, and KPE samples (i.e. Estuarine group); the second included the DJR, MDR, CLR, and TWR samples (i.e. West-South-reservoir group), and the third included the SMR (i.e. North-reservoir group). The results of randomisation test show that it is unlikely that the extremely low misclassification rates from the original dataset are a result of chance alone. Therefore, there are significant morphological divergences between the three groups. Animals with the same morphometric measurements are often assumed to constitute a stock (Waldman et al., 1988; Giri and Collins, 2004; Collins et al., 2007; Giri and Loy, 2008; Torres et al., 2014). If a stock is considered

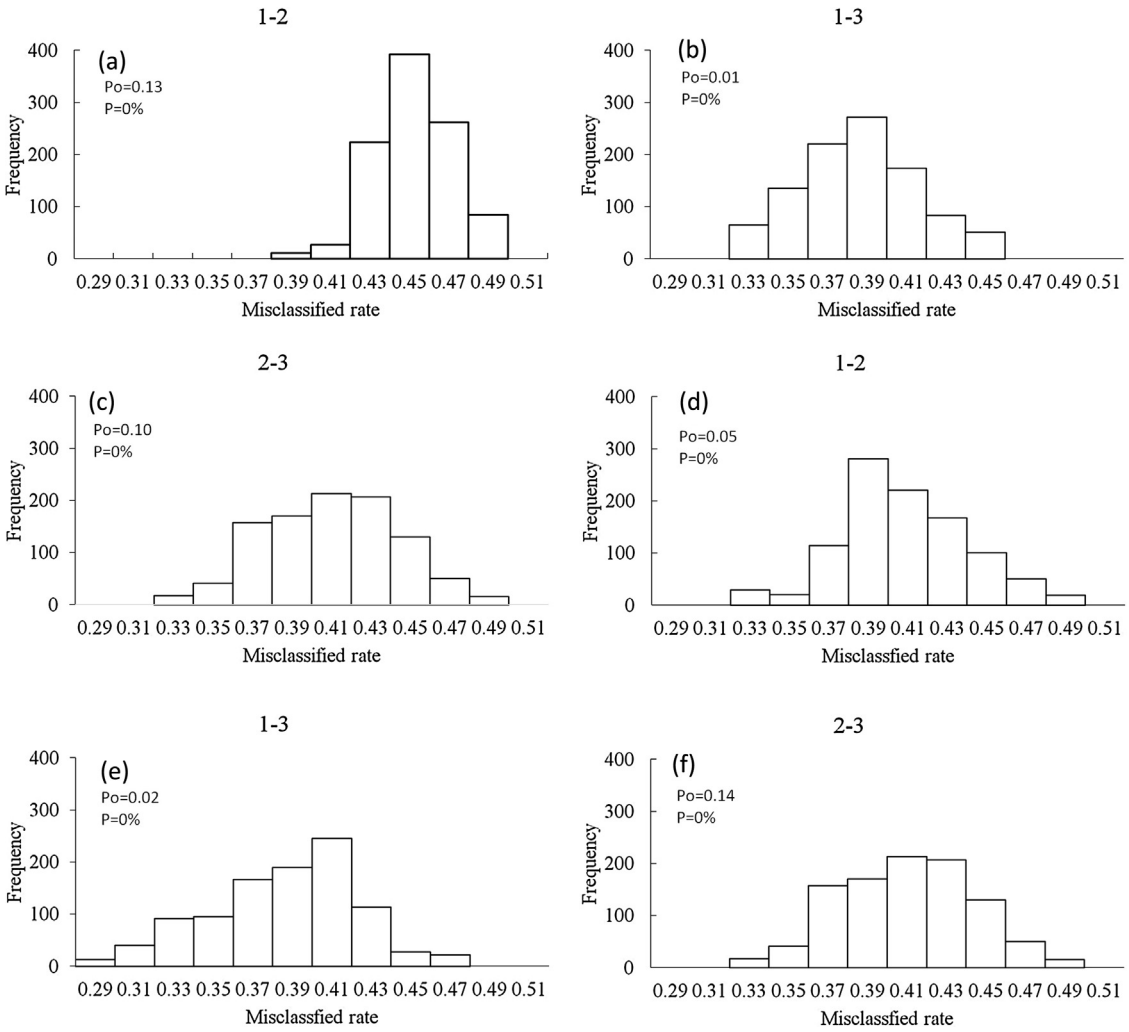

Fig. 6. Frequency distribution of 1000 misclassification rate (Pc) estimated from (a) the the WS-reservoir group and the Estuarine group for female, (b) the WS-reservoir group and the N-reservoir group for female, (c) the Estuarine group and the N-reservoir group for female, and (d) the WS-reservoir group and the Estuarine group for male, (e) the WS-reservoir group and the N-reservoir group for male, (f) the Estuarine group and the N-reservoir group for male, *P*, proportion of Pc < Po among 1000 permutations; Po, misclassification rate estimated from the original dataset.

an intraspecific group of individuals exhibiting unique phenotypic attributes (Tzeng et al., 1998; Tzeng and Yeh, 1999; Mashiko and Numachi, 2000; Tzeng, 2004), the results of the present study indicate at least three morphologically distinguishable stocks of *M. nipponense* in Taiwan.

Although morphometric studies have been proven valuable in providing insight into the discrimination of marine stocks, several factors may confound the analytical results of a morphological relationship between geographically distinct populations (e.g. sexual dimorphism, timing of sampling, and allometric growth) (Kinsey et al., 1994; Giri and Collins, 2004; Collins et al., 2007; Giri and Loy, 2008; Torres et al., 2014). The present paper attempts to minimise variances caused by these parameters through using the size correction technique, approaching sampling times, narrowing the differences in sizes between samples, and statistical analyses by sex. Restricting sample comparisons to specific length classes might disregard ontogenetic changes within samples, which could be necessary for meaningful descriptions of morphometric differences (Bookstein et al., 1985). However, this effect might not be serious in the present study, because there is a small range of carapace lengths in each sample. Size-related characteristics play a significant role in morphometric analysis, and the results maybe erroneous if not adjusted for statistical data analyses (Tzeng and Yeh, 1999; Tzeng, 2004; Sajina et al., 2011; Torres et al., 2014). Morphological differences are solely related to body shape variation if the effects of size are successfully removed (Tzeng and Yeh, 2002; Anvarifar et al., 2011). In the present study, we successfully removed the size effect by allometric transformation of the data. Therefore, the significant differences between the eight samples of the oriental river prawn in Taiwan can be attributed to variations in body shape. Results also indicate that there were significant differences among study sexes and sites (Table 4).

The significant morphometric variation between the WS- and N-reservoir groups is of particular interest. In Taiwan, previous studies have shown that the phylogeographic patterns of freshwater fish usually indicate a close relationship between populations of the west-central and northern regions (Wang et al., 1999; Tzeng, 1986; Wang et al., 2007). However, the present results show that populations of the oriental river prawn of the west-central and southern regions are closely related, as indicated by two freshwater fish (Wang et al., 2000; Wang et al., 2004) and one fresh prawn (Liu et al., 2011). This may have been caused by two incursive colonisation scenarios, through which species migrated from China to Taiwan. One migratory route entails dispersal into northern Taiwan; the other migratory route entails an initial dispersal through the west-central region and a subsequent dispersal northward and southward (Liu et al., 2011). The Miaoli Plateau formed a potential geographical barrier preventing gene flow between the

two colonisation events (Tzeng, 1986). To form and maintain discrete stocks, a moderately high degree of reproductive isolation is essential (Horrall, 1981).

Significant morphometric variation between reservoir and estuarine populations were also found, and that could be improved by the differences in fishery biology between these two populations. Mashiko (1983a) indicated that the estuarine group spawns a large number of small eggs, and the freshwater group spawns a small number of large eggs. The growth of upper freshwater individuals was noticeably depressed, but most of the females seemed to spawn at one year of age, which is similar to the estuarine females during nearly the same breeding season (Mashiko, 1983b). The body sizes of females at maturity were significantly smaller in populations inhabiting fresh waters than those found in populations inhabiting brackish-water lakes (Mashiko, 2000).

No significant difference was found among the estuarine samples in this paper. The migratory distance of the oriental river prawn in estuary was limited (Mashiko and Numachi, 1993, 2000). Therefore, the dispersal of larvae often play important role in reducing variation between stocks or populations. Ocean currents, therefore, play a major role in the dispersal of a species. For the oriental river prawn, it took 28 days for zoea larvae to metamorphose into juveniles (Wu and Tang, 1990). A portion of the Kuroshio Current flows into Taiwan Strait along the south-western coast of Taiwan, and the water masses of the South China Sea flow through the Taiwan Strait into the East China Sea (Fig. 1). The three sampling areas were primarily exchange overlaid by Kuroshio water masses and the water masses of the South China Sea.

Conclusion

This study was to use multivariate statistical techniques to analyse and compare the morphometric differences of *Macrobrachium nipponense*, and at least three morphologically distinguishable stocks in Taiwan are obtained. There were also significant differences between estuarine and freshwater populations in Taiwan. No significant morphometric difference was found between estuarine samples in this paper, and ocean currents may play important role in reducing variation between these three samples. There were significant differences between reservoir samples. Particularly, the sample from Shimen Reservoir (the N-reservoir group) is evidently different from the rest reservoir samples (WS-reservoir group), and that may be derived from different evolutionary origins or geographic events. The current results and discussions here are essential to be further verified using molecular techniques in the future.

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